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Formulation and Evaluation of niosome containing Glycolic acid for acne dark spots

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ABSTRACT

A Non-Ionic surfactant-based vesicles, otherwise called niosomes, stand outenough to be noticed in drug fields because of their amazing conduct in typifying both hydrophilic and hydrophobic specialists. As of late, it has been found that these vesicles can work on the bioavailability of medications, and may work as another technique for conveying a few normal of remedial specialists, for example, chemical and protein drugs and gene materials with low poisonousness and wanted to focus on efficiency. Compared with liposomes, niosomes are significantly steadier during the plan interaction and storage. The required pharmacokinetic properties can be accomplished by streamlining part sort by surface modification. This original conveyance framework is likewise simple to get ready and scale up with low creation costs. In this thesis, we sum up the formulation, method, and characterization of niosome and its applications in skin diseases like Acne dark spots. Niosomal gel containing drug is being formulate for effective delivery of drug into the skin as gel has good absorption rate. The method and evaluations of gel is we sum up in this thesis.

Keywords: Niosome; Cholesterol; Hydrophilic and Lipophilic drugs; Surfactant; Targeted delivery; Bioavailability; Applications; Niosomal Gel.

1. Introduction

1.1. Niosome

NIOSOMES, which are formed with non-ionic amphiphiles in specific fluid arrangements through self-gather innovation, were first utilized in improvement of beauty care products. With the advancement of nanoscales in the field of pharmaceutics, an ever increasing number of studies have zeroed in on non-ionic surfactants as nano particle carriers for drug conveyance. Un-ionized surfactant can be an option in contrast to liposome's and polymeric vesicles because of their capacity to typify various types of medications to build their dependability and adequacy. Dissimilar to other nanoparticles, primarily, liposome's, polymeric vesicles and niosomes have numerous similitudes, and they can be there in every way stacked with both aquaphilic and aquaphobic medications. Along these lines, they could co-convey both hydrophilic and hydrophobic medications in a single vesicle. Non-ionic surfactants, which form niosomes, are more chemically and physically stable than lipids, making them much more stable than lipids.

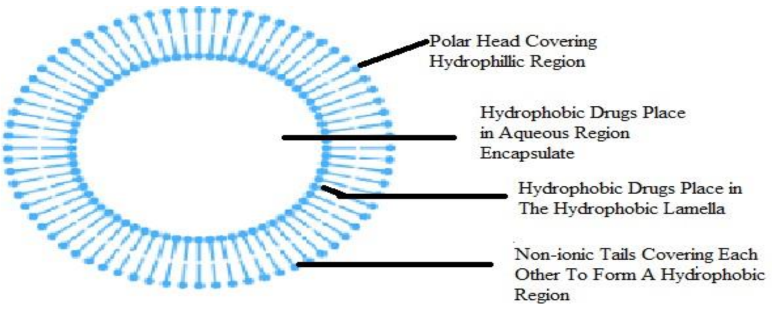


Figure 1.1. NIOSOME

1.2. Study Objectives

- To perform a preformulation study of drug identification of drug, solubility studies, and FTIR studies.

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

- To optimize the formula by preparing different batches of niosomes containing glycolic acid and preparing different trial formulations.
- To formulate final batches of niosome contacting glycolic acid using suitable methods and study different evaluation parameters.
- To perform stability test.
- To perform tests for the performance of glycolic acid incorporated in niosome.

2. Material and Method

S. No.	Material
1.	Cholesterol
2.	Water
3.	Span 80
4.	Tween 80
5.	Ethanol
6.	Glycolic Acid
7.	Sodium Phosphate
8.	Sodium Phosphate Monobasic
9.	H_2O
10.	Carbapole
11.	Triethnol amine

2.1. Solubility analysis

Preformulation solubility analysis was done, which include the selection of a Suitable solvent to dissolve Glycolic Acid.

2.2. Preparation of Phosphate Buffer pH 6.5

By dissolving 1.35 g of Potassium Dihydrogen Phosphate, 2.4 g of Disodium Hydrogen Phosphate and 7.1 g of Sodium chloride in Distilled water for up to 1000 ml. This formulation provides the Phosphate Buffer of pH 6.5. We can adjust the pH if needed.

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

2.3. Preparation of Niosome

Weighed Quantity of Cholesterol + Surfactant was taken

Organic solvent was added to the above

The drugs solution were added to the thin film

A thin layer of film was formed in a round bottom flask

The niosomes was formed and characterized

Figure 2.3. Flowchart for Preparation of Niosome

Table 2.3. Preparation of Niosome

Material	**F1**	**F2**	**F3**	**F4**	**F5**
Drug	5gm	5gm	5gm	5gm	5gm
Cholesterol	2.5gm	2.5gm	2.5gm	2.5gm	2.5gm
Tween 80	5gm	5gm	-	-	5gm
Span80	5gm	5gm	5gm	-	5gm


2.4. Preparation of Niosomal Gel

1% w/w Carbopol 940 was dissolved in distilled water to create the gel basis, which was then left to swell for an hour. Glycerin was then continuously homogenized and added to the dispersion. Triethanolamine was used to change the pH.

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

2.5. Preparation of niosome loaded glycolic acid gel

Carbopol (1.5%), Glycerol (10%), Triethanolamine (q. s.), and distilled water up to 15g were used to make the gel base. The promising niosome suspension (a preparation of niosomes preparing by using the refine ratio of surface active agent and glycolic acid equal to 2 percent w/w was introduced into the gel base.

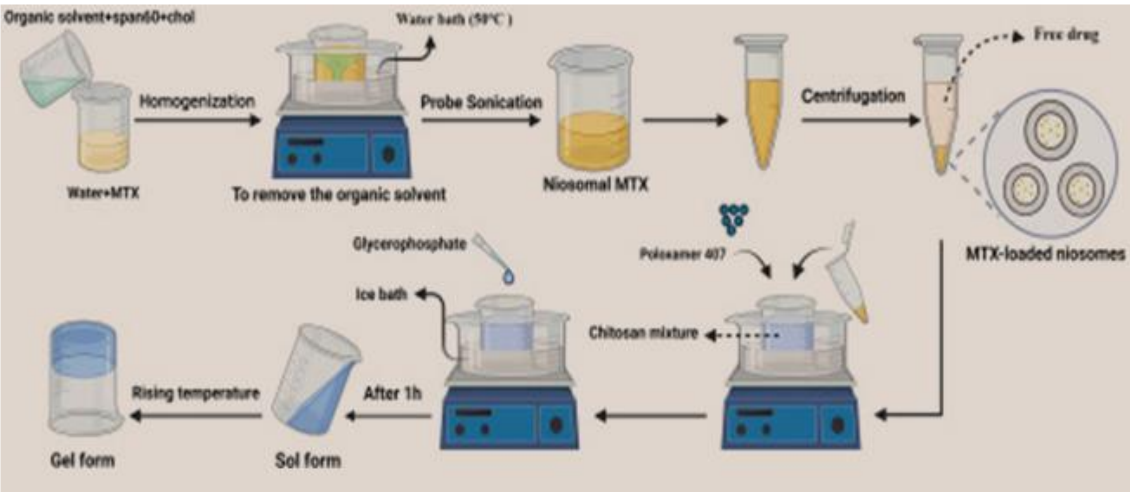


Figure 2.5. Diagram - Niosomal Gel Preparation

Table 2.5. Niosomal Gel preparation

Material	F1	F2	F3	F4
Niosome (5mg/ml)	2ml	2ml	3ml	2ml
Carbapole	1gm	1gm	.1gm	1gm
Phosphate Buffer 6.5 pH	10ml	10ml	10m	10ml
Glycerin	100mg	150mg	200mg	250mg
Triethnol amine	2-4 drops	3-4	4-5	5-6

Figure 2.5.1. Picture of Niosomal Gel preparation

2.6. Evaluation of Niosomal gel

2.6.1. pH of gel

A pH meter was used to measure the formed gel's pH.

2.6.2. Measuring viscosity

It was a digital viscometer made by Brookfield. Spindle No. 7 was used to measure the gel's viscosity.

2.6.3. The formulation's appearance

Grittiness, color, consistency, and odor were all measured visually.

2.6.4. The Capability to Spread

The spreadability of the gel was evaluated by sandwiching a sample of the gel mixture between two glass slides. A weight is attached to the slide above. Glass slides were dragged or slipped on the bottom glass slides based on the weight in the pan.

The formula below was then utilized to determine spreadability:

$M \times L/T = S$

The spread ability is denoted by S = the weight of the pan (attached to the upper slide) is M.

T is the amount of time in seconds, and L is the length that the glass slide moved.

2.6.5. The capacity to extrude

The 0.5 mm hole was completed and the prepared gels were poured into collapsible tubes. The formulation's extrudability has been verified. Where ++ good, +++ exceptional, and + average.

2.6.6. % of the drug

"Take 1 gram of gel." Blend it with an appropriate solvent. To get a clear solution, filter it. Utilizing a UV spectrophotometer, find its absorption. By utilizing the same standard plot and entering the absorbance value into the standard plot equation, the concentration and drug content were calculated.

2.6.7. Stability investigation

"Studies were conducted at 40° C ± 2°C and 75% ± 5% relative humidity (RH) using prepared gels packaged in aluminum collapsible tubes (5 g).Samples were removed at one-month intervals and assessed for drug concentration, dissemination ability, pH, and physical appearance.

2.6.8. Drug Release in Vitro

Diffusion Study for Niosomes: The in vitro release of glycolic acid from the Niosome formulations was examined using the open-ended cylinder technique. This diffusion cell device uses a glass tube that is open on both ends and has an inner diameter of 2.5 cm. One end is connected to an artificial membrane and serves as a donor compartment.

This investigation's goal is to determine the penetration rate. Data from in vitro experiments and the amount of time needed to reach a steady state permeation flow were used to optimize the formulations. Studies of drug release from Niosomal gel formulations were carried out using the in vitro diffusion technique for a 24-hour period at 38 °C and 100 rpm.

2.6.9. Study on skin irritation in vitro

Hen's Egg Test on the Chorioallantoic Membrane, or HET-CAM.

Resources: White Hen's Eggs fresh (within the last seven days), productive.

Weight: 50 to 60 grams.

Incubator setting up the test system: 50–60 g eggs were found to be fertile.

The egg is incubated for nine days: Throughout the first eight days of incubation, eggs should be turned at least five times a day.

One more day of incubation without turning. After removing the outer white cell, the inner membrane was moistened with a 0.9% (w/v) NaCl solution. Before using for 20 minutes, remove the inner membrane.

Utilizing the test substance for treatment: Direct application of the test material to the CAM. CAM exposure to the test material for a minimum of three minutes.

Endpoint determined by ocular examination:

Haemorrhage: Red blood specks surrounding the CAM blood vessels as they bleed out.

Lyesis: The CAM cave's optical vanishing of tiny blood vessels. Based on general pathologic principles, this is not a true Lyesis.

Blood coagulation: Denaturation of albumin, extravascular blood coagulation (dark spots), and thrombosis (intravascular dark spots).

2.7. Result and Discussion

2.7.1. Solubility studies

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

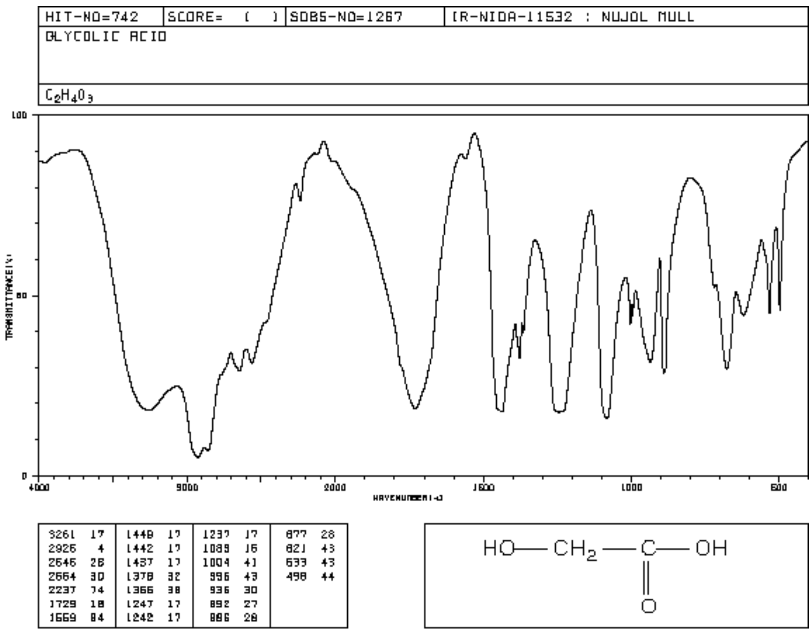
Table 2.7.1. Solubility

S. No.	Chemical Name	Solubility
1.	Methanol	+ + + + +
2.	Ethanol	+ + + +
3.	Ether	+ + +
4.	Water	+ + +

2.7.2. Drug excipients interaction studies

The drug and the excipients namely carbopol, Cholesterol were analyzed by Fourier-Transform I.R. spectrophotometer.

The FT-IR spectrums were interpreted and it is shown there is no interaction between the drugs with the excipients was conformed.



Glycolic acid

Table 2.7.2. Interpretation of FTIR of Glycolic acid

S. No.	Peak Positions	Groups
1.	3261.17	O-H stretching, O-H stretching
2.	2926.4	O-H stretching, N-H stretching, C-H stretching
3.	2545.26	O-H stretching

4.	1729.18	C-H bending, C=O stretching
5.	1669.64	C=O stretching, N-H stretching, C=N stretching, C=C stretching
6.	1449.19	O-H stretching, C-H stretching
7.	1442.1	C-H stretching, C-H stretching
8.	1457.17	C-H stretching, C-H stretching
9.	1378.32	O-H stretching, S=O stretching, C-F stretching, C-N stretching
10.	1366.38	C-F stretching, C-N stretching, S=O stretching
11.	1247.17	C-F stretching, C-N stretching, S=O stretching

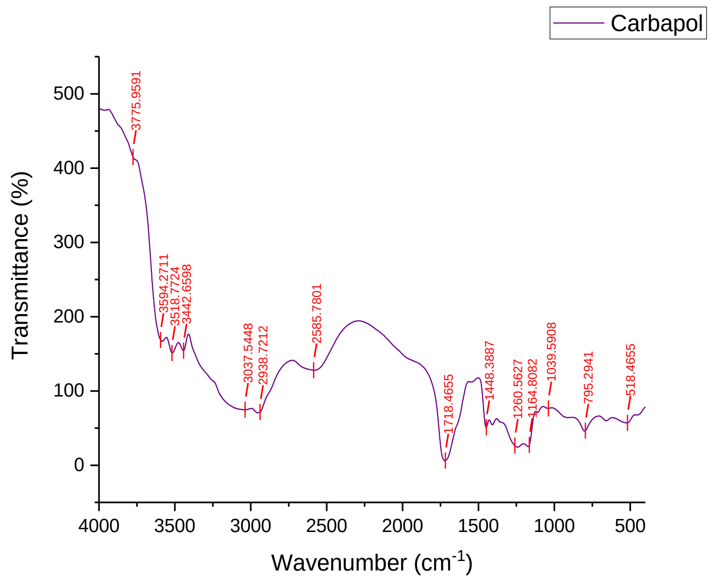


Table 2.7.3. FT-IR interpretation of Carbapol

S. No.	Peak Position	Group
01	3594.2711	O-H stretching
02	3518.7724	O-H stretching, N-H stretching
03	3442.6598	O-H stretching
04	3037.5448	O-H stretching, C-H stretching
05	2938.7212	O-H stretching, N-H stretching, C-H stretching

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

06	2585.7801	O-H stretching, S-H stretching
07	1718.4655	C-H bending, C=O stretching
08	1448.3887	C-H bending
09	1260.5627	C-F stretching, C-O stretching
10	1164.8082	C-F stretching, C-N stretching, C-O stretching
11	1039.5908	C-F stretching, C-N stretching, S=O stretching
12	795.2941	C-Cl stretching, C=C bending, C-H bending
13	518.4655	C-Br stretching, C-I stretching

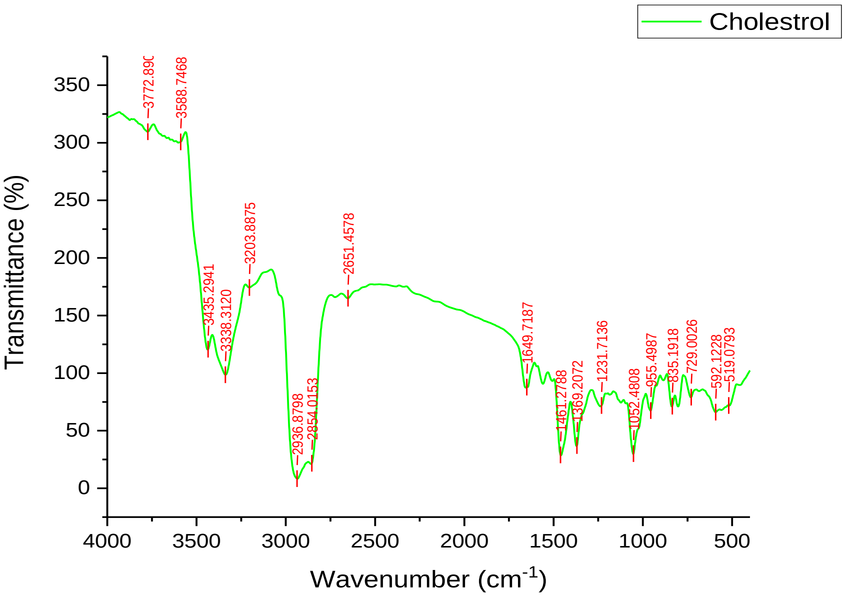


Table 2.7.4. Interpretation of FTIR of Cholesterol

S. No.	Peak Position	Group
1.	34352941	O-H stretching
2.	33383120	O-H stretching, N-H stretching, N-H stretching
3.	34003875	O-H stretching, N-H stretching
4.	29945838	O-H stretching, N-H stretching, C-H stretching
5.	29740153	O-H stretching, O-H stretching N-H stretching, C-H stretching

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

6.	29514578	O-H stretching, O-H stretching, N-H stretching, C-H stretching
6.	16497187	C=O stretching, N-H stretching, C=N stretching, C=C stretching
7.	14312788	O-H stretching, C-H stretching
8.	13882478	O-H stretching, C-F stretching, C-H stretching, S=O stretching
9.	13332002	O-H stretching, S=O stretching, C-F stretching, C-N stretching
10.	1231.7135	C-F stretching, C-O stretching, C-N stretching
11.	1052.4303	C-N stretching, C-F stretching, S=N stretching

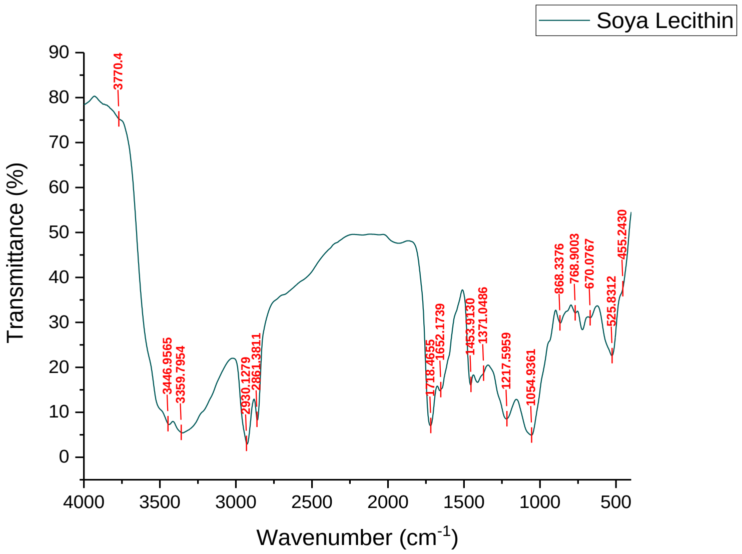


Table 2.7.5. FTIR interpretation of soya lecithin

S. No.	Peak Position	Group
01	3446.9565	O-H stretching
02	3359.7954	O-H stretching, N-H stretching
03	2930.1279	O-H stretching, N-H stretching, C-H stretching
04	2861.3811	O-H stretching, N-H stretching, C-H

		stretching
05	1718.4655	C-H bending, C=O stretching
06	1652.1739	C-H bending, C=O stretching, C=N stretching, C=C stretching
07	1453.9130	C-H bending
08	1371.6486	O-H bending, C-F stretching, S=O stretching
09	1217.5959	C-F stretching, C-O stretching, C-N stretching
10	1034.9361	C-F stretching, C-N stretching, S=O stretching
11	868.3376	C-H bending
12	768.9003	C-Cl stretching, C-H bending
13	670.6767	C-Cl stretching, C=C bending, C-Br stretching
14	525.8312	C-Br stretching, C-I stretching

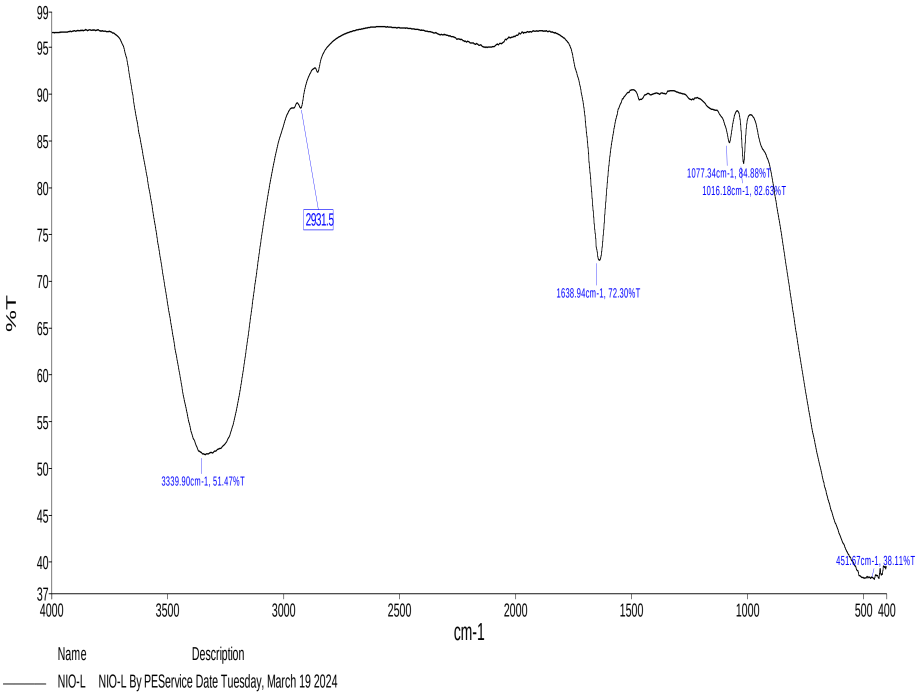


Table 2.7.6. FTIR interpretation of NIO-L

S. No.	Peaks	Groups
1.	3339.90	O-H stretching, N-H stretching, N-H stretching

Asian Journal of Basic Science & Research
Volume 6, Issue 2, Pages 125-144, April-June 2024

2.	2931.5	O-H stretching, N-H stretching, C-H stretching
3.	1638.94	C=O stretching, N-H stretching, C=C stretching
4.	1077.34	C-F stretching, C-N stretching, C-O stretching
5.	1016.18	C-F stretching
6.	451.67	-

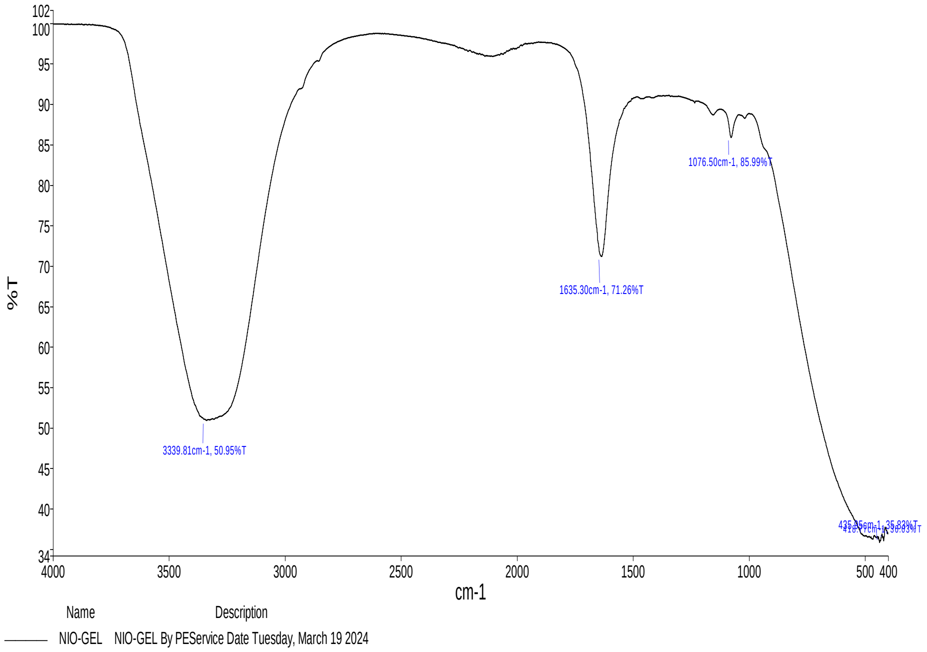


Table 2.7.7. FTIR of NIO-GEL

S. No.	Peaks	Groups
1.	3339.81	O-H stretching, N-H stretching, N-H stretching
2.	1635.30	C=O stretching, N-H stretching, C=C stretching
3.	1076.50	C-F stretching, C-N stretching, C-O stretching

2.8. Particle size and Morphology

Niosomal formulations shape and morphology were ascertained through optical microscopy. Microscopic examination revealed spherical large uni-lamellar vesicles of 450-560nm size range. The average mean particle size of formulation-2 was 510nm, respectively.

2.8.1. Size Distribution of Niosomes

Niosomes were subjected in to laser particle counter (L.P.C) for characterizing size distribution of niosomes. Its shows that the particle size range 450-580nm range. The average means particle size of formulation 3,574nm respectively.

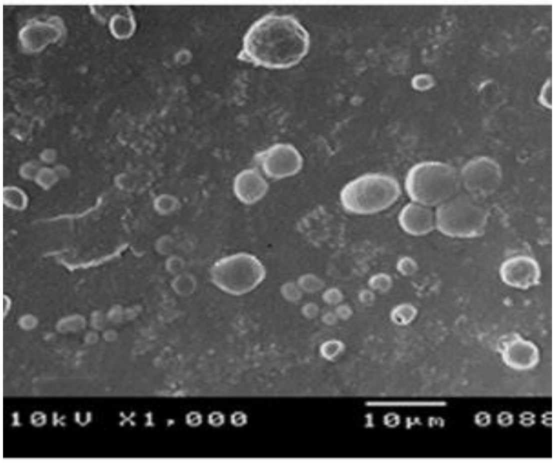


Figure 2.8.1. SEM of Niosome

2.8.2. Electron Microscopy for Transmission (TEM)

By using TEM examination, the morphological features of the niosomal formulations were further verified. The spherical shape of niosomes was shown by a TEM photomicrograph of (N2) niosomal formulation at 18,000x magnification. Furthermore, the hollow vesicular structure of niosomes was noted from the TEM images a, b, c, d.

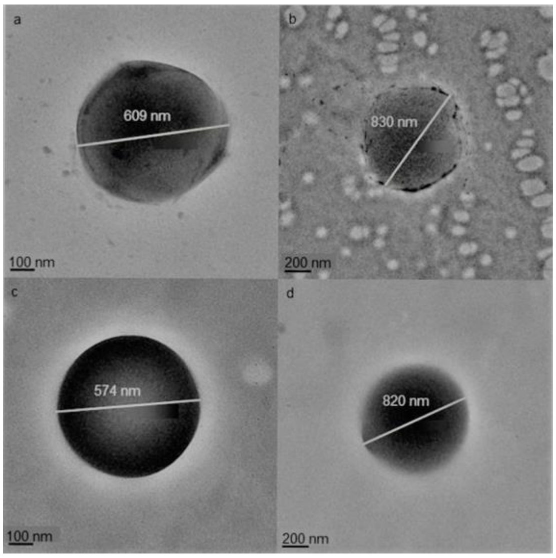


Figure 2.8.2. TEM image of Niosome

2.9. Evaluation of Niosomal Gel

2.9.1. Physical Appearance

"Gel formulations were white preparation with a smooth homogeneous texture and glossy appearance. Results have been discussed in Table 2.9.1.

Table 2.9.1. Results

S. No.	Formulation	Colour	Phase separation	Homogeneity
1	F1	White	None	Excellent
2	F2	White	None	Excellent
3	F3	White	None	Excellent
4	F4	White	None	Excellent
5	F5	White	None	Excellent

2.9.2. pH of Gel

The pH of the Gel formulation was in the range of 3.0 – 4.0 which considered acceptable to avoid the risk of skin irritation upon application to skin.

Table 2.9.2. pH

Formulation	F1	F2	F3	F4	F5
pH	3.4	3.5	3.0	3.8	3.9

2.9.3. Determination of viscosity

The viscosity of the gels was determined by using Brookfield viscometer. The viscosity of the formulations was ranged from 30,000 to 80,000 cps and the results were shown in Table 2.9.3.

Table 2.9.3. Viscosity

S. No.	Formulations	Values in cps
1.	Formulation 1	30,000 cps
2.	Formulation 2	30,300 cps
3.	Formulation 3	41,900 cps
4.	Formulation 4	61,200 cps
5.	Formulation 5	1,20,550 cps

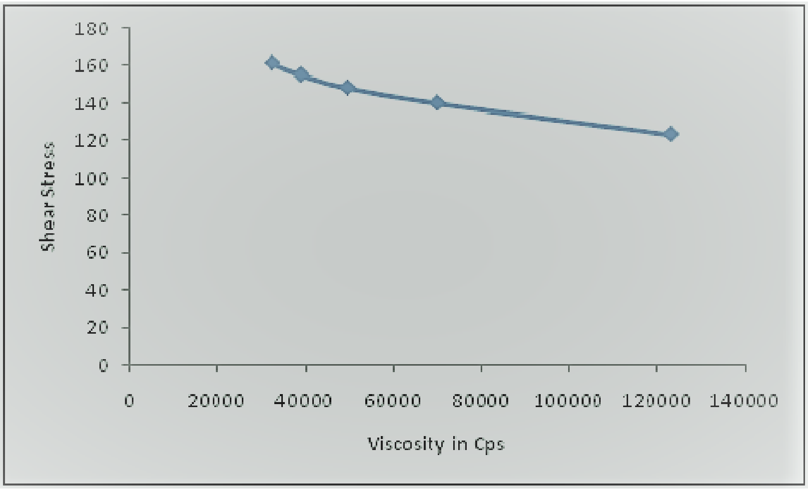


Figure 2.9.3. Viscosity

2.9.4. Extrudability

The formulated gels was filled into collapsible tubes and 0.5mm hole is done. The extrudability of the formulation has been checked. Where + average, ++ good, +++ excellent. As shown in the Table 2.9.4.

Table 2.9.4. Extrudability

S. No.	Formulations	Extrudability
1.	Formulation 1	+ + +
2.	Formulation 2	+ + +
3.	Formulation 3	+ + +
4.	Formulation 4	+ +
5.	Formulation 5	+ +

2.9.5. Spreadability

Spreadability of the plain gel and Niosomal gel formulation were found to be better as compared to plain.

Table 2.9.5. Spreadability

S. No.	Formulation	Result
01	Formulation-01	21±35
02	Formulation-02	24±35
03	Formulation-03	25±35
04	Formulation-04	22±35
05	Formulation-05	28±35

2.10. Diffusion Study

In Vitro Drug Release

Diffusion Study for Transfersomes

Table 2.10. Drug Release Test

S. No.	Time in (hrs)	Absorbance at 337 nm
1	0	0
2	0.25	0.627
3	0.5	0.648
4	0.75	0.656
5	1	0.659
6	1.5	0.662
7	2	0.668
8	2.5	0.669
9	3	0.671
10	4	0.674
11	5	0.678
12	6	0.686
13	7	0.688
14	8	0.689
15	9	0.694
16	10	0.696
17	11	0.698
18	12	0.699
19	14	0.703
20	16	0.709
21	18	0.715
22	20	0.717
23	22	0.718
24	24	0.718

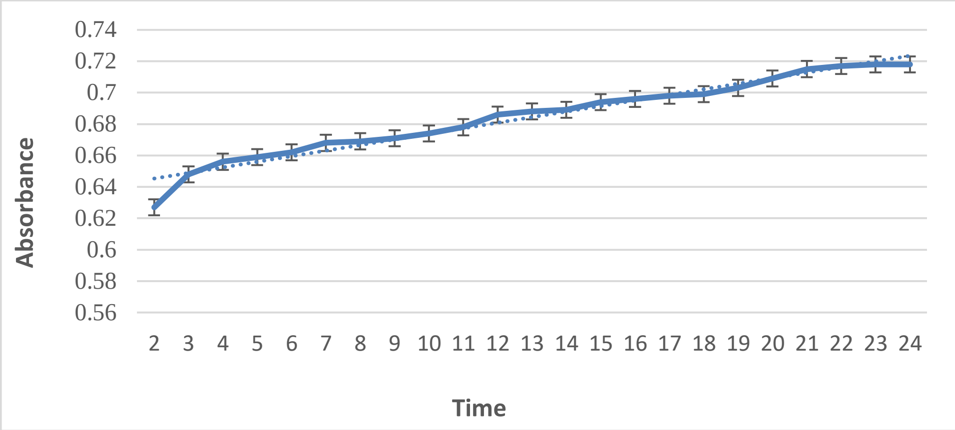


Figure 2.10. Drug Release Test

Table 2.10.1. Skin Irritation Test

End Point	Observation
Hemorrhage	No
Lysis	No
Coagulation	No

Figure 2.10.1. Egg Membrane

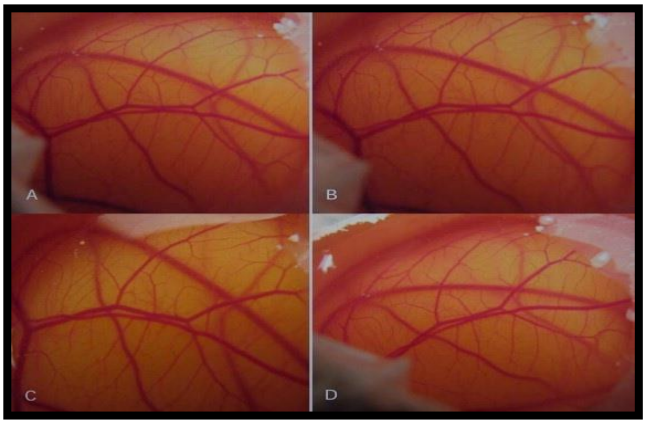


Figure 2.10.2. Egg Membrane

2.11. Stability Studies

The stability studies of Niosomal formulation were carried out at refrigeration temperature (4 °C), Room temperature and 40 °C. Physical evaluation of prepared Niosomal gel shown in the Table 2.11.

Table 2.11. Physical evaluation of prepared Niosomal gel

Parameter	**Room temperature (25 ^{0}C)**	**40 ^{0}C**	**4 ^{0}C**
Appearance			
Initial	White colour gel	White colour gel	White colour
1 month	White colour gel	White colour gel	White colour
2 month	White colour gel	White colour gel	White colour
3 month	White colour gel	White colour gel	White colour
pH Initial	3.0	3.0	3.0
1 month	3.0	3.0	3.0
2 month	3.5	3.5	3.5
3 month	3.5	3.5	3.5
Viscosity			
Initial	30,000 cps	30,000 cps	30,000cps
1 month	30,300 cps	30,300 cps	30,300 cps
2 month	41,900 cps	41,900 cps	41,900 cps
3 month	61,200 cps	61,200 cps	61,200 cps
Extrudability			
Initial	Satisfactory	Satisfactory	Satisfactory
1 month	Satisfactory	Satisfactory	Satisfactory
2 month	Satisfactory	Satisfactory	Satisfactory
3 month	Satisfactory	Satisfactory	Satisfactory
Phase Separation			
Initial	Not found	Not found	Not found
1 month	Not found	Not found	Not found
2 month	Not found	Not found	Not found
3 month	Not found	Not found	Not found
Texture Initial	Smooth	Smooth	Smooth
1 month	Smooth	Smooth	Smooth
2 month	Smooth	Smooth	Smooth
3 month	Smooth	Smooth	Smooth

3. Conclusion

Effective treatment of acne, acne marks, Pustules and Scars, hyper pigmentation can be done by Glycolic acid. Utilization of proper polymers such as Carbopol was providing better drug release, better stability and good patient compliance. All the Excipients are compatible with acne and scars, hyper pigmentation it also enhance permeability of Glycolic Acid which helps to treat Scars and hyper pigmentation. Niosomal gel containing glycolic acid has good drug absorption and penetration rate into the dermal layers. As gel get absorb fast and effective. Glycolic acid present in the gel has pH range 3-4% which is effective to treat hyper pigmentation, scars, and dark spots as it breaks the bond in skin and improves cells turnovers in the skin first layer and safe to use every day as skin tolerance.3-4% is safe and has no or less side effects and can be used at home. In this thesis we used this percentage to make this drug effective and safe to treat conditions without causing many side effects.

4. Future Prospects

Niosomes symbolize a capable drug release molecule. There is a bunch of possibility to encapsulate toxic anti-infective drug, anti-inflammatory drug, anti-cancer drug, anti-aids drug, etc. in niosome to use them as capable drug carrier to achieve better targeting and bioavailability properties and for sinking the toxic and side-effects of the drugs. The ionized drug carrier is comparatively toxic and incompatible while niosome containing carrier is safer. Managing and storage space of niosomes need no out of the ordinary conditions

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis and manuscript writing equally.

Availability of data and material

All data pertaining to the research is kept in good custody by the authors.

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